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(54) Title: COMPOSITIONS AND METHODS FOR TRANSDUCTION OF CELLS

(57) **Abstract**

A method of transducing proliferating cells to a novel phenotype by administering to the cells an amount effective to transduce at least a portion of the target cells of a composition wherein the active agent is a nucleotide molecule including at least one sequence corresponding to a cell lineage commitment gene and compositions for use in the method are described. Conversion of non-myocytes (for examples, fibroblasts in the ischemic heart) to the skeletal muscle phenotype is effected by injection of a vector expressing a muscle regulatory factor gene. Cellular conversion through exogenous MyoD expression demonstrates the potential of converting one type of cell (for example, the areas of fibrotic tissue within the ischemic heart wall) to another (e.g., a skeletal muscle phenotype). Such tragedies lead to the development of alternative therapeutic interventions in a variety of conditions, including those involving injured or traumatized tissue (e.g., muscle in ischemic heart disease).

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COMPOSITIONS AND METHODS FOR TRANSDUCTION OF CELLS

Background of the Invention

The present invention relates generally to the fields of biotechnology and medicine. More particularly, the present invention is directed to compositions and methods useful in the transduction of cells for therapeutic purposes.

Considerable research is currently being directed to techniques for somatic gene therapy, in which a therapeutic gene encoding a therapeutic protein is delivered to cells, tissues or organs manifesting a disease. One therapeutic approach involves introduction of a suitable vector containing the gene *ex vivo* into autologous cells from the site at which the therapy is directed, followed by reimplantation of the cells. Alternatively, introduction of genetic material directly into mammalian cells may be effected *in vivo* through the use of, e.g., viral particles functioning in the ordinary course of infection; retroviruses have been found to be especially useful as vectors for accomplishing gene insertion.

Several strategies have been developed to use skeletal muscle tissue to influence the performance of a failing heart. Skeletal muscle grafts with neuronal innervation have been used clinically as a means of strengthening the contractions of the ischemic heart. Such approaches are subject to the limitations imposed by surgical intervention and post operative side effects.

One possible way to circumvent these problems would be to transplant skeletal muscle cells into the myocardium in the hopes that they might fuse and form functional fragments of contractile tissue. Experimental attempts to insert such myogenic cells recently has been attempted in animal models, leading to the formation of a patch of well demarcated skeletal muscle surrounded by normal myocardium [Koh, G.Y. et al. (1993) *Am. J. Physiol*, **264**, H1727-H1733].

Gene transfer into the normal myocardium has been achieved through direct DNA injection, resulting in high level and long term expression from cell type specific, hormone responsive and ubiquitously active promoters [Lin, H. et al. (1990) *Circ.*, **82**, 2217-2221; Kitsis, R. N., et al. (1991) *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **88**, 4138-42;

Acsadi, G. et al. (1991) *New Biol.*, 3, 71-81; von Harsdorf, R. et al. (1993) *Circulation Research*, 72, 688-95]. It had not heretofore been determined, however, whether directly injected foreign genes are transcribed in ischemic myocardium.

It is an object of the present invention to provide compositions and methods useful for the therapeutic transduction of cells. In particular, it is an object of the present invention to provide compositions and methods which may be employed in the treatment of injured or diseased muscle.

Summary of the Invention

In accordance with the present invention, delivery of a nucleotide sequence corresponding to a cell lineage commitment gene is employed to transduce proliferating cells to a novel phenotype. For example, proliferating fibroblasts are transduced to a myogenic phenotype as a technique for remediation of muscle injury or trauma. In a particular embodiment of the invention, delivery of muscle regulatory factor gene to the myocardium is employed in order to convert to a myogenic phenotype the cells that populate a myocardial scar following infarction. Upon conversion of the proliferating cardiac fibroblasts to skeletal muscle cells, they strengthen the weakened heart wall, effect the tissue remodeling that accompanies post-infarction processes, and potentially contribute to contraction. The feasibility of this approach is demonstrated by direct introduction of a myogenic determination gene, MyoD1, into the scar tissue of an experimental model of myocardial infarction.

Detailed Description of the Invention

In accordance with the present invention, it has now been demonstrated both *in vivo* and in cell culture that it is possible to convert non-myocytes, for example fibroblasts in the ischemic heart, to the skeletal muscle phenotype by injection of a vector expressing a muscle regulatory factor gene. These observations on cellular conversion through exogenous MyoD expression indicate the potential of converting one type of cell (for example, the areas of fibrotic tissue within the ischemic heart wall) to another (e.g., skeletal muscle phenotype) by delivery to the target cells of at least one nucleotide sequence corresponding to a cell lineage

commitment gene. Such strategies lead to the development of alternative therapeutic interventions in a variety of conditions involving injured or traumatized tissue (e.g., muscle in ischemic heart disease).

The present invention calls for the introduction of at least one nucleotide sequence corresponding to or functionally equivalent to (as hereinafter defined) a mammalian gene which regulates cell lineage commitment into a suitable proliferating cell. One type of gene of particular interest for use in accordance with the present invention is the family of muscle regulatory factor genes. The muscle regulatory factor gene family includes, but is not limited to, the following genes: myogenin [Edmondson, D.G. & Olson, E.N. (1989) *Genes & Development*, **3**, 628-640]; Myf-5 [Braun, T. et al. (1989) *EMBO Journal*, **8**, 701-709]; MRF4 [Rhodes, S.J. & Konieczny, S.F. (1989) *Genes & Development*, **3**, 2050-2061]; and MyoD1 [Weintraub, H. et al. (1991) *Science*, **251**, 761-766; Davis, R.L. et al. (1987) *Cell*, **51**, 987-1000].

MyoD1 has been shown to convert non-muscle cells to the skeletal muscle phenotype *in vitro* [Weintraub, H. et al. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 5434-5438; Choi, J. et al. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 7988-92]. Members of this family of genes act as transcription factors capable of transactivating muscle specific genes [Lassar, A.B. et al. (1989) *Cell*, **58**, 823-831], and display cell cycle suppressive activities [Crescenzi M. et al. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 8442-6; Sorrentino V. et al. (1990) *Nature*, **345**, 813-5]. Conversion of chondroblasts, epithelial, nerve, fat and fibroblast cells has been demonstrated in cell culture to varying degrees [Weintraub et al. (1989), *supra*; Choi et al., *supra*]. When expression of exogenous MyoD in cardiocytes is forced during development in transgenic mice, there is an hypertrophic response and conversion to the skeletal muscle phenotype [Miner, J.H. et al. (1992) *Development*, **114**, 853-860]. However, it had not heretofore been established whether such phenotypic changes could be induced by introduction of a muscle regulatory factor gene into adult tissues *in vivo*.

The reported sequence for a MyoD cDNA [Davis et al., *supra*] is as follows:

ACACCTCTGACAGGACAGGACAGGGAGGAGGGGTAGAG
GACAGCCGGTGTGCATTCCAACCCACAGAACCTTTGTCAT
TGTACTGTTGGGGTTCCGGAGTGGCAGAAAGTTAAGACG
ACTCTCACGGCTTGGGTTGAGGCTGGACCCAGGAACTGG
GATATGGAGCTTCTATCGCCGCCACTCCGGGACATAGA
CTTGACAGGCCCCGACGGCTCTCTCTGCTCCTTTGAGA
CAGCAGACGACTTCTATGATGATCCGTGTTTCGACTCA
CCAGACCTGCGCTTTTTTGAGGACCTGGACCCGCGCCT
GGTGCACGTGGGAGCCCTCCTGAAACCGGAGGAGCAC
GCACACTTCTCTACTGCGGTGCACCCAGGCCCAGGCG
CTCGTGAGGATGAGCATGTGCGCGCGCCCAGCGGGCA
CCACCAGGCGGGTCGCTGCTTGCTGTGGGCCTGCAAG
GCGTGCAAGCGCAAGACCACCAACGCTGATCGCCGCA
AGGCCGCCACCATGCGCGAGCGCCGCCGCCTGAGCAA
AGTGAATGAGGCCTTCGAGACGCTCAAGCGCTGCACG
TCCAGCAACCCGAACCAGCGGCTACCCAAGGTGGAGA
TCCTGCGCAACGCCATCCGCTACATCGAAGGTCTGCAG
GCTCTGCTGCGCGACCAGGACGCCGCGCCCCCTGGCG
CCGCTGCCTTCTACGCACCTGGACCGCTGCCCCCAGG
CCGTGGCAGCGAGCACTACAGTGGCGACTCAGACGCG
TCCAGCCCGCGCTCCAACTGCTCTGATGGCATGATGGA
TTACAGCGGCCCCCCAAGCGGCCCCCGGCGGCAGAAT
GGCTACGACACCGCCTACTACAGTGAGGCGGTGCGCG
AGTCCAGGCCAGGGAAGAGTGCGGCTGTGTCGAGCCT
CGACTGCCTGTCCAGCATAGTGGAGCGCATCTCCACA
GACAGCCCCGCTGCGCCTGCGCTGCTTTTGGCAGATG
CACCACCAGAGTCGCCTCCGGGTCCGCCAGAGGGGGC
ATCCCTAAGCGACACAGAACAGGGAACCCAGACCCCG
TCTCCCGACGCCGCCCCTCAGTGTCCTGCAGGCTCAAA
CCCCAATGCGATTTATCAGGTGCTTTGAGAGATCGACT
GCAGCAGCAGAGGGCGCACCACCGTAGGCACTCCTGGGG

ATGGTGCCCCTGGTTCTTCACGCCCAAAAGATGAAGCTTA
AATGACACTCTTCCCAACTGTCCTTTCGAAGCCGTTCTTC
CAGAGGGAAGGGAAGAGCAGAAGTCTGTCCTAGATCCAG
CCCCAAAGAAAGGACATAGTCCTTTTTGTTGTTGTTGTTG
TAGTCCTTCAGTTGTTTGTTTGTTTTTTCATGCGGCTCACA
GCGAAGGCCACTTGCACTCTGGCTGCACCTCACTGGGCC
AGAGCTGATCCTTGAGTGGCCAGGCGCTCTTCCTTTCCTC
ATAGCACAGGGGTGAGCCTTGCACACCTAAGCCCTGCCC
TCCACATCCTTTTGTTTGTCACTTTCTGGAGCCCTCCTGG
CACCCACTTTTCCCCACAGCTTGCGGAGGCCACTCAGGTC
TCAGGTGTAACAGGTGTAACCATACCCCACTCTCCCCCTT
CCCGCGGTTCAGGACCACTTATTTTTTTATATAAGACTTT
TGTAATCTATTCGTGTAAATAAGAGTTGCTTGGCCAGAGC
GGGAGCCCCTTGGGCTATATTTATCTCCCAGGCATGCTGT
GTAGTGCAACAAAAACTTTGTATGTTTATTCCTCAAGCGG
GCGAGTCAGGTGTTGGAAATCC [SEQ ID NO:1].

It is further reported that the sense orientation encodes only one open reading frame that is greater than 100 residues and has a good initiation sequence consensus (GATATGG); this portion of the molecule is indicated in bold face.

The reported sequence for a myogenin cDNA [Edmondson et al., *supra*] is as follows:

GGTCNNNNCTACAGAGGCGGGGGCGGGCCCAGCCCATGG
TGCCCAGTGAATGCAACTCCCAGGGGCCCTCNNCTGCGG
GACGTTGGGGGCCAGTGGCAGGAACAAGCCTTTTGCGAC
CTGATGGAGCTGTATGAGACATCCCCCTATTTCTACCA
GGAGCCCCACTTCTATGATGGGGAAAACTACCTTCCTG
TCCACCTTCAGGGCTTCGAGCCCCCGGGCTATGAGCG
GACTGAGCTCAGCTTAAGCCCGGAAGCCCGAGGGCCC
CTGGAAGAAAAGGGACTGGGGACCCCTGAGCATTGTC
CAGGCCAGTGCCTGCCGTGGGCATGTAAGGTGTGTAA
GAGGAAGTCTGTGTCGGTGGACCGGAGGAGGGCAGCC

ACACTGAGGGAGAAGCGCAGGCTCAAGAAAGTGAATG
AGGCCTTCGAGGCCCTGAAGAGGAGCACCCTGCTCAA
CCCCAACCAGCGGCTGCCTAAAGTGGAGATCCTGCGC
CATGCCATCCAGTACATTGAGCGCCTACAGGCCTTGCT
CAGCTCCCTCAACCAGGAGGAGCGCGATCTCCGCTAC
AGAGGCGGGGGCGGGCCCAGCCCATGGTGCCCAGTGA
ATGCAACTCCCACAGCGCCTCCTGCAGTCCGGAGTGG
GGCAATGCACTGGAGTTCGGTCCCAACCCAGGAGATC
ATTTGCTCGCGGCTGACCCTACAGACGCCCACAATCTG
CACTCCCTTACGTCCATCGTGGACAGCATCACGGTGGA
GGATATGTCTGTTGCCTTCCCAGACGAAACCATGCCCA
ACTGAGATTGTCTGTCAGGCTGGGTGTGCATGTGAGC
CCCCAAGTTGGTGTCAAAAGCCATCACTTCTGTAGCAG
GGGGCTTTTAAGTGGGGCTGTCCTGATGTCCAGAAAACA
GCCCTGGGCTGCCACAAGCCAGACTCCCCACTCCCCATT
CACATAAGGCTAACACCCAGCCCAGGGAGGGAATTTAGC
TGACTCCTTAAAGCAGAGAGCATCCTCTTCTGAGGAGAG
AAAGATGCAGTCCAGAGAGCCCCCTTGTTAATGTCCCTC
AGTGGGGCAAACTCAGGAGCTTCTTTTTTGTTTATCATAT
ATGCCTCGAATTCCACCCCCCACCCCCAAAATGAAACCG
TTTGAGAGACATGAGTGCCCTGACCTGGACAAGTGTGCA
CATCTGTTCTAGTCTCTTCCTGAAGCCAGTGGCTGGGCTG
GGCCTGCCCTGAGTTGAGAGAGAAGGGGGAGGAGCTATC
CGGTTCCAAAGCCTCTGGGGGCCAAGCATTTGCAGTGGA
TCTTGGGNNNNTTCCAGTGCTTTGTGTATTGTTTATTGTT
TTGTGTGTTGTTTGTAAAGCTGCCGTCTGCCAAGGTCTCC
TGTGCTGATGATACCGGGAACAGGCAGGCCAGGGGGTGG
GGGCTCTTGGGGTGACTTCTTTTGTTAACTAAGCATTGTG
TGGTTTTGCCAATTTTTTTTCTTTTGTAATTCTTTTGCTAA
CTTATTTGGATTTCCTTTTTTAAAAAATGAATAAAGACTG

GTTGCTATCAGAAAAAAAAAAAAAAAAAAAAAAA

[SEQ ID NO:2].

Once again, the reported longest uninterrupted ORF is indicated in bold.

The following nucleotide sequence for MRF4 cDNA has been reported [Rhodes & Konieczny, *supra*]:

AGTCCATCACCCAGTTCAGATCAGTCAGAGGCCAAGGAG
GAGAACATGATGATGGACCTTTTTGAAACTGGCTCCTA
TTTCTTCTACTTAGATGGAGAAAATGTGACTCTTCAGC
CATTAGAAGTGGCAGAGGGCTCTCCTTTGTACCCAGG
GAGTGATGGTACCCTATCCCCTTGCCAGGACCAAATGC
CCCAGGAAGCCGGGAGCGACAGCAGTGGAGAGGAACA
CGTTCTGGCTCCCCCAGGCCTTCAGCCACCCCACTGCC
CAGGTCAGTGTCTGATCTGGGCTTGCAAGACTTGCAAG
AGAAAATCTGCCCCCACAGATCGTCGGAAAGCAGCTA
CCCTGCGCGAAAGGAGGAGGCTTAAGAAAATCAACGA
AGCCTTTGAGGCCTTGAAGCGTAGAACTGTGGCCAAC
CCCAACCAGAGGCTGCCCAAGGTGGAGATTCTGAGAA
GTGCCATCAACTACATTGAGCGTCTGCAGGACCTGCTG
CACCGGCTGGATCAGCAAGAGAAAATGCAGGAGCTGG
GGGTGGACCCTTACAGCTACAAACCCAAGCAAGAAATT
CTTGAGGGTGCGGATTTCCTGCGCACCTGCAGCCCGC
AGTGGCCAAGTGTTTCGGATCATTCCAGGGGCCTGGT
GATAACTGCTAAGGAAGGAGGAGCAAGCGTCGATGCT
TCAGCCTCCAGCAGTCTTCAGCGCCTTTCTTCCATCGT
GGACAGTATTTCCTCAGAGGAACGCAAACTCCCCAGC
GTGGAGGAGGTGGTGGAGAAGTAACTCAGTCAGCATTT
GGAACATTCTTCGCTCAGCAGGAAGAGCCCCTTTCCGCCT
AATCATTTAGATTAGGGCTCACAGACCCCAGAATTTATGA
AAGGCAAGAGACTTAGTGTTAAAAAAGAAACCTCTCCCC
ACCTCAAGTGAAAATCCTTCGGCTTGGGGCTTTTATTATA
ACTATTATTGTATCTGAACCGCTAGTGGCTTAGCTCTAGA

ACCCTAATTTTGTTTTTAGTTTGGTTGGTTTTTTATAACAT
ATTAACTTTTGCTATGATCACGTGACCCTTTCCTGTCAGT
TGCAAACGAAGTTCATTCCTGTCTAAATCAAAGTGGGAA
CGTTTAATCCTAAGAGTATTTAATGTACTTTTGTAAATAG
TCTTAGTACTTTCGTTTTTATGTAAACCTAAAGGACATAT
TTTAAATGTGGAATTAAGCATTGTATATAAAATGTGTGAG
AGTCTGATATTGTAATATTAAAATATTAATACGTTTCTAC
ACGTAAAAAAAAAAAAAAAAAAAAAAAAA [SEQ ID NO:3].

The single major ORF is indicated in bold.

A Myf-5 cDNA sequence has been reported [Braun et al., *supra*] as follows:

CCTCTCGCTGCCGTCCAGGTGCACCGCCTGCCTCTCAGCA
GGATGGACGTGATGGATGGCTGCCAGTTCTCACCTTCT
GAGTACTTCTACGACGGCTCCTGCATACCGTCCCCCGA
GGGTGAATTTGGGGACGAGTTTGTGCCGCGAGTGGCT
GCCTTCGGAGCGCACAAAGCAGAGCTGCAGGGCTCAG
ATGAGGACGAGCACGTGCGAGCGCCTACCGGCCACCA
CCAGGCTGGTCACTGCCTCATGTGGGCCTGCAAAGCC
TGCAAGAGGAAGTCCACCACCATGGATCGGCGGAAGG
CAGCCACTATGCGCGAGCGGAGGCGCCTGAAGAAGGT
CAACCAGGCTTTCGAAACCCTCAAGAGGTGTACCACGA
CCAACCCCAACCAGAGGCTGCCCAAGGTGGAGATCCT
CAGGAATGCCATCCGCTACATCGAGAGCCTGCAGGAG
TTGCTGAGAGAGCAGGTGGAGAACTACTATAGCCTGC
CGGGACAGAGCTGCTCGGAGCCCACCAGCCCCACCTC
CAACTGCTCTGATGGCATGCCCGAATGTAACAGTCCTG
TCTGGTCCAGAAAGAGCAGTACTTTTGACAGCATCTAC
TGTCCTGATGTATCAAATGTATATGCCACAGATAAAAA
CTCCTTATCCAGCTTGGATTGCTTATCCAACATAGTGG
ACCGGATCACCTCCTCAGAGCAACCTGGGTTGCCTCTC

CAGGATCTGGCTTCTCTCTCTCCAGTTGCCAGCACCGA
TTCACAGCCTCGAACTCCAGGGGCTTCTAGTTCCAGGC
TTATCTATCATGTGCTATGAACTAATTTTCTGGTCTATAT
GACTTCTTCCAGGAGGGCCTAATACACAGGACGAAGAAG
GCTTCAAAAAGTCCCAAACCAAGACAACATGTACATAAA
GATTTCTTTTCAGTTGTAAATTTGTAAAGATTACCTTGCC
ACTTTATAAGAAAGTGTATTTAACTAAAAAGTCATCATTG
CAAATAATACTTTCTTCTTCTTTATTATTCTTTGCTTAGAT
ATTAATACATAGTTCCAGTAATACTATTTCTGATAGGGGG
CCATTGATTGAGGGTAGCTTGTTCGAATGCTTAACTTATA
TATACATATATATATATTATAAATATTGCTCATCAAAATG
TCTCTGGTGTTTAGAGCTTTATTTTTTTCTTTAAAACATTA
AAACAGCTGAGAATCAGTTAAATGGAATTTTAAATATATT
TAACTATTTCTTTTCTCTTTAATCCTTTAGTTATATTGTAT
TAAATAAAAATATAATACTGCCTAATGTATATATTTTGAT
CTTTTCTTGTAAGAAATGTATCTTTTAAATGTAAGCACAA
AATAGTACTTTGTGGATCATTTCAAGATATAAGAAATTTT
GGAAATTCCACCATAAATAAAATTTTTTACTACAAGAAAAA
[SEQ ID NO:4].

The single ORF in both orientations as reported is indicated in bold.

In the retroviral mediated gene therapy experiments in ischemic heart models reported herein, proliferating fibroblasts represent the most likely target cell. This is based on a) the propensity of retroviral transduction for proliferating cells and b) the observation that following ischemic injury in adult myocardium the cardiomyocytes do not proliferate.

The strategy of using at least one muscle regulatory factor gene to convert cells in a cardiac scar *in vivo* is useful in strengthening the contractions of the injured heart and preventing the deleterious consequences of myocardial remodeling following infarction. Thus, the present invention is particularly useful in the treatment of heart muscle that is weak or functioning poorly. A prime situation for use of the inventive protocol is the treatment of ischemic heart tissue resulting from

coronary artery disease or coronary infarction. Other pathologic abnormalities resulting in muscle which is weak or functioning poorly (such as cardiomyopathy, hypertensive heart disease or long-standing valvular disease) may also be ameliorated in accordance with the inventive method.

Moreover, the ability to deliver genes such as MyoD to organs and tissues by various delivery vectors could have more widespread use than the heart model described in detail herein. For example, the present invention has a clear utility in the conversion of non-muscle cells to skeletal muscle phenotype in repair of other injured or ischemic muscles, including but not limited to muscles of the eye, hand and foot. MyoD was the first identified mammalian gene that appears to regulate cell lineage commitment. It is likely that other such genes exist that are master switches for other tissue types (such as nerves, skin, bone and cartilage). As such genes are characterized, they are also suitable for use in accordance with the present invention for therapeutic conversion.

The present invention involves the administration of therapeutic compositions useful in the transduction of proliferating cells to a novel phenotype, as well as in delivering a therapeutic nucleotide sequence corresponding to a cell lineage commitment gene to those cells. Exemplary nucleotide sequences corresponding to cell lineage commitment genes for use in accordance with the present invention include members of the muscle regulatory factor gene family or functional equivalents thereof. By a "functional equivalent" of a muscle regulatory factor gene is intended a nucleotide sequence or portion thereof encoding a peptide corresponding to a product of a member of the muscle regulatory factor gene family or a portion of such a peptide sufficient to achieve the desired transduction of proliferating cells to a myogenic phenotype. Although various reported cDNA sequences for members of the muscle regulatory factor gene family are disclosed herein, also contemplated as clearly within the scope of the present invention are variant forms of the heretofore-identified muscle regulatory factor genes and functional equivalents thereof, including sequences containing mutations and deletions, which are competent to encode a peptide which achieves the desired cell transduction. In particular, those portions of the reported cDNA sequences

identified as encoding the corresponding muscle regulatory factor, degenerate sequences encoding these factors, and sequences encoding peptides which are functionally equivalent to these factors in transducing target cells would be immediately recognized by those skilled in the art as equivalents and thus well within the scope of the present invention. Similar considerations apply with respect to other cell lineage commitment genes and nucleotide sequences corresponding to cell lineage commitment genes.

In addition, preferred nucleotide sequences include an active constitutive or inducible promoter sequence as are well known in the art. Conventional engineered vector constructs, such as plasmid and bacteriophage (phage) vectors, containing suitable promoter sequences are useful in accordance with the present invention. These and other DNA sequences which are able to replicate in a host cell may be employed in accordance with the present invention as cloning vehicles in a manner well known in the art. The therapeutic nucleotide sequence of the present invention may suitably comprise a DNA construct capable of generating therapeutic nucleotide molecules in high copy numbers in the target cells, as described in published PCT application WO 92/06693, the entire disclosure of which is hereby incorporated by reference. Further, a wide variety of known retroviral vectors may also be employed in accordance with the present invention. Transduction with viral vectors has been valuable as a tool for obtaining high level gene expression in a high proportion of cells within a target area [Nabel, E.G. et al. (1989) *Science*, **244**, 1342-4; Nabel, E.G. et al. (1991) *Journal Of The American College Of Cardiology*, **17** 189B-194B]. Retroviral vectors preferentially infect dividing cells and for this reason are not useful to transduce a terminally differentiated cell type, such as cardiomyocytes. They would, however, be expected to transduce proliferating cardiac fibroblasts *in vivo* following ischemic injury.

Recent DNA transfer techniques employing uptake of recombinant adenovirus by normal myocardium [Stratford-Perricaudet, L.D. et al. (1992) *Journal of Clinical Investigation*, **90**, 626-30] may also be particularly applicable to myocytes following ischemia and reperfusion in accordance with the present

invention. Recombination of such vectors is rare; there are no known associations of human malignancies with adenoviral infections despite common human infection with adenoviruses; the genome may be manipulated to accommodate foreign genes of a fairly substantial size (up to about 7.5 kb in length); live adenovirus has been safely used as a human vaccine; and host proliferation is not required for expression of adenoviral proteins.

In various embodiments of the present invention, therapeutic compositions useful for practicing the therapeutic methods described herein are contemplated. Therapeutic compositions of the present invention may contain a physiologically acceptable carrier together with one or more therapeutic nucleotide sequences as described herein, dissolved or dispersed in the carrier, as the active ingredient. In preferred embodiments of the invention, the composition is not immunogenic or otherwise able to cause undesirable side effects when administered to a mammal or human patient for therapeutic purposes.

As used herein, the term "physiologically acceptable" as applied to compositions, carriers, diluents and reagents, represents that the materials are capable of administration to or upon a mammal, including a human patient, without the production of undesirable physiological effects, including but not limited to nausea, dizziness, gastric upset and the like.

The preparation of a pharmacological composition which contains active ingredients dissolved or dispersed therein is well understood in the art. Typically, such compositions are prepared for purposes of injection as liquid solutions or suspensions; however, solid forms suitable for solution or suspension in liquid prior to use may also be prepared.

Physiologically acceptable carriers are well known in the art. Exemplary liquid carriers for use in accordance with the present invention are sterile aqueous solutions which contain no materials other than the active ingredient and water, or may contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both (i.e., phosphate-buffered saline). Suitable aqueous carriers may further comprise more than one buffer salt, as well as other salts (such as sodium and potassium chlorides) and/or other solutes.

The active ingredient may further be mixed in amounts suitable for use in the therapeutic methods described herein with one or more excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include dextrose, glycerol, ethanol and the like, and combinations of one or more thereof with vegetable oils, propylene glycol, polyethylene glycol, benzyl alcohol and the like to provide a suitable injectable composition. In addition, if desired, the composition can contain wetting or emulsifying agents, isotonic agents, pH buffering agents, dissolution promoting agents, stabilizers, antiseptic agents and other typical auxiliary additives employed in the formulation of pharmaceutical preparations. In another variation, the therapeutic nucleotide sequences of the present invention may be incorporated into liposomal vesicles [see, e.g., U.S. Patent 5,104,661; U.S. Patent 5,013,556; and published PCT application WO 92/06192, the entire disclosures of which are hereby incorporated by reference].

A therapeutic composition for use in accordance with the present invention typically contains an amount of the therapeutic nucleotide sequence as described herein sufficient to deliver a therapeutically effective amount to the target tissue. Following the protocols described herein, at least about 10% of the target cells in an infarcted area are transduced upon administration of a therapeutic composition in accordance with the present invention, and preferably about 20% to about 30% are affected. It is anticipated that with the use of more powerful vectors, transduction of at least 50% of the targets cells can be achieved. For purposes of the present invention, it is contemplated transduction of at least a portion of the target cells corresponding to about 10% thereof is sufficient to achieve a desired therapeutic effect. Typically, the compositions comprise at least about 0.1 weight percent to about 90 weight percent of therapeutic nucleotide sequence per weight of total therapeutic composition.

The therapeutic nucleotide compositions comprising synthetic oligonucleotide sequences in accordance with the present invention may be prepared in a manner known per se to those skilled in the art by suitable method, including but not limited to the phosphotriester and phosphodiester methods, as

described in, e.g., Narang et al., *Meth. Enzymol.* 68:90 (1979), Brown et al., *Meth. Enzymol.* 68:109 (1979) and U.S. Patent 4,356,270.

The method of the present invention generally comprises contacting specific cells with a therapeutically effective amount of a pharmaceutically acceptable composition comprising a therapeutic nucleotide sequence of this invention. This contact may suitably be effected by a direct injection of the compositions into tissues or organs comprising the target cells. Alternatively, for treatment of ischemic tissue intracoronary administration may be effected via a catheter. Finally, intravenous administration of suitable compositions may be employed.

Whereas skeletal muscle grafted onto heart by cardiomyoplasty is innervated [Hooper, T.L. et al. (1993) *Surgery Annual*, 1, 157-73] non-myocytes converted to skeletal muscle with MyoD may require electrical stimulation if they are to contract. Thus, to effectively repair damage cardiac tissue the conduction system of the newly-created skeletal muscle must be coupled to that of the healthy cardiac tissue. The electrical currents present in the cardiac tissue could be sufficient to induce coupling to the adjacent skeletal muscle tissue. Alternatively, exogenous transfer of cardiac gap junction protein [Beyer, E.C. et al. (1987) *Journal of Cell Biology*, **105**, 2621-9; Fishman, G. I. et al. (1990) *Journal of Cell Biology*, **111**, 589-98; Beyer, E.C. et al. (1990) *Journal of Membrane Biology*, **116**, 187-94] by gene transfer would permit coupling between these different muscle cell types. To stimulate sympathetic innervation, exogenous expression of the gene for nerve growth factor (NGF) within the converted myotubes may lead to creation of a conduction system within the skeletal muscle tissue.

A further consideration in attempts to ensure the conversion of scar to contractile muscle within the heart is the lack of vascularization of the infarcted region. To solve this problem exogenous expression of angiogenic factors such as acidic and basic FGF by gene transfer [Barr, E. et al. (1991) *Circulation*, **84 (Suppl II)**, II-420] could lead to neovascularization within the ischemic area. To obtain sufficient levels of these foreign proteins within newly formed skeletal muscle careful attention must be paid to the use of appropriate promoter/enhancer systems that will ensure high level and long term gene expression. For this

purpose striated muscle specific promoters whose specificity was validated *in vivo* would be suitable choices. Alternatively, if these foreign genes were introduced into unconverted cardiac fibroblasts a strong promoter would be appropriate to give high level gene expression.

The invention may be better understood with reference to the accompanying examples, which are intended for purposes of illustration only and should not be construed as in any sense limiting the scope of the present invention as defined in the claims appended hereto.

Examples

The plasmid RSV (Rous Sarcoma Virus)-luciferase containing the luciferase gene under the control of the Rous Sarcoma Virus promoter is described in R. Kitsis et al., *supra*. Amphitrophic retroviruses containing the MyoD coding sequence were obtained as a supernatant from PA317 cells. First, 2 ml of retroviral supernatant derived from LMDSN infected ψ cells was used to transduce PA317 cells at a density of 5 x 10^5 cells/100 mm dish. Infection was conducted for 3 hours after which the culture medium was changed. 24 hours later cells were split 1:400 for selection in G418 containing medium (1.5 mg/ml) and cultured until antibiotic resistant colonies were visible. One such colony was used as a source of MyoD encoding amphotrophic retrovirus.

To test whether functional MyoD protein was expressed by the amphitrophic retrovirus, 2 ml of retroviral supernatant was used to infect 10T1/2 cells at 5 x 10^5 cells/ 100 mm dish. Cells were infected for 3 hours and transferred to fresh medium. 24 hours later cells were split 1:20 for selection in G418 containing medium. After 3 weeks in culture approximately 100 colonies/dish were formed. These colonies contained cells that differentiated into an extensive array of multinucleated myotubes which confirmed that functional MyoD was expressed by the amphitrophic retrovirus.

β-galactosidase encoding retrovirus was obtained from a PA317 clone transduced with LNPOZ. The viral titer of LNPOZ was 5 x 10^5 colony forming units per ml.

For preparation of retroviral supernatants (LMDSN or LNPOZ) transduced PA317 cells were grown to a density of 5 x 10^5 cells/100 mm dish and cultured in the absence of G418 for 24 hours after which supernatants were moved and stored at -80°C. The LMDSN vector is described in Weintraub et al. (1989), *supra*.

Tissue was homogenized with a Polytron (Kinematic, Switzerland) for 45 sec in 1 ml of ice cold homogenization buffer [Brasier, A.R. et al. (1989) *BioTech.*, **7**, 1116-1122]. For measurements of luciferase activity samples were assayed for peak light production at 10 sec with a Monolight model 2001 luminometer (Analytical Luminescence Laboratory). 100 μl of each homogenate was combined with 350 ml of Buffer B and 100 μ of the buffer containing D-luciferin.

To assay for β-galactosidase, 3 mm sections of tissue were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) for 10 min at room temperature and incubated for 2 hours at 37°C in 1 mM 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal), 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM $MgCl_2$. Samples were then embedded in OCT compound and 10 μ frozen sections were cut and counterstained with nuclear fast red.

Photomicroscopy was performed using Scotchrome 1000 film and a Zeiss microscope. Immunostaining of frozen tissue sections for skeletal specific MHC performed using monoclonal antibody MY-32 (Sigma) at 1:400 dilution and anti-chicken α-actinin antiserum (Sigma) at 1:500 dilution. Staining was visualized using fluorescein conjugated goat anti-mouse immunoglobulin G (skeletal MHC) and rhodamine conjugated goat anti-rabbit immunoglobulin G (α-actinin).

For determination of collagen content in infarcted areas tissue was fixed in formalin and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin and picrosirius red.

Example 1

Cell Culture

Neonatal rat cardiac fibroblasts were isolated according to the literature procedure [Simpson, P. et al. (1982) *Cir. Res.*, **50**, 101-116]. Heart tissue was finely minced and subjected to gentle trypsinization and mechanical disruption for

4-5 hours. Disassociated cells were plated for 30-60 minutes on 100 mm tissue culture dishes, after which the culture medium containing the cardiomyocytes was removed. Adherent cardiac fibroblasts were then expanded in culture for retroviral transduction. Cardiac fibroblasts were plated in 60 mm dishes with 5 x 10^5 cells/dish and polybrene was added at 8 μg/ml. Cells were infected with 100 μl of retroviral supernatant for 2 days. The cells were detached by trypsinization and seeded at densities ranging from 300 to 60,000 cells per plate. The cells were grown in the presence of G418 (800 μg/ml) and the medium changed every 3-4 days. After 12 days in culture G418 resistant colonies were isolated.

After transduction of the primary neonatal rat cardiac fibroblasts with either LMDSN carrying the MyoD and neomycin resistance genes or LMDSN carrying only the neomycin resistance gene, cells resistant to G418 were compared. The morphology of the LMDSN transduced cells was distinctive compared to that of cells transduced with vector alone. The former had been converted to a markedly extended shape and formed elongated multinucleated cells compared to the rounded fibroblast morphology retained by the control cells. Only the LMDSN transduced cells reacted positively with an antibody specific to the skeletal muscle-specific isoform of myosin heavy chain. Thus, primary cardiac fibroblasts can be converted to the skeletal muscle phenotype by overexpression of MyoD.

Example 2

Rat Model of Myocardial Ischemia and Reperfusion

Female Sprague Dawley rats were anesthetized with 0.1 ml/100 g (body weight) of ketamine (50mg/ml) and xylazine (10mg/ml) injected intra-peritoneally and the chests were surgically opened to expose the beating heart. The coronary artery was occluded as described [Li, Y. et al. (1992) *Cardiovascular Research*, **26**, 226-31] for 15 (n=12) or 60 minutes (n=6) followed by reperfusion. DNA injections (1-3 per rat) were performed 15 minutes later, after which the chest was closed. The DNA solution contained 50 μg of pRSVLuc and 1% (vol/vol) Evans blue dye. 11 rats served as sham, non ischemic controls for the 60 minute group. Animals were sacrificed 7 days after surgery and hearts were removed for analysis of luciferase activity.

Whereas genes delivered by direct heart injection into normal hearts enter myocardial cells and are expressed, it had not been determined whether ischemic or otherwise injured myocardium would also be capable of accepting and expressing exogenously delivered DNA. Accordingly a short (15 minute) and a long (60 minute) interval of myocardial ischemia followed by reperfusion were used in order to engender different degrees of injury to the myocardium, which was then followed by direct injection of reporter genes. Hearts were excised 7 days after the injection to determine whether the injected gene was expressed. The results are presented in Table 1. Since the number of DNA injections varied from animal to animal the absolute values of luciferase activity observed in the injected hearts can be taken only as an indication of whether ischemic myocardium can process and robustly express exogenous genes and cannot be used to compare between sham and ischemic groups.

High level RSV-luciferase reporter gene activity was observed after delivery of exogenous DNA to the ischemic area of occluded hearts. The peak light production from these mildly ischemic heart samples did not differ from the levels obtained in non ischemic control animals. Significant levels of luciferase expression above 100 light units (measured over 10 seconds) were obtained with ten of 12 rats in the ischemia/reperfusion group and nine of 11 rats in the non-

ischemic control group. Rats that failed to express likely did so because of faulty injection. One hour occlusion with 7 days of reperfusion also resulted in a high level of gene expression. There was a trend (not statistically valid) toward greater expression in this ischemic/reperfusion group compared to the non-ischemic group. Five of 6 rats in the non-ischemic control group and all 6 rats in the ischemic group expressed significant amounts of luciferase activity.

Example 3

Production of Myocardial Infarction in Dogs

Mongrel dogs were anesthetized with sodium pentobarbital (35 mg/kg), intubated, and ventilated. Myocardial infarcts were created percutaneously by embolizing alpha helix thrombotic coils (Target Therapeutics) into the left anterior descending coronary artery, under fluoroscopic guidance. Angiography was repeated at approximately 20-40 minutes to confirm coronary occlusion.

Six to 11 days after coronary occlusion the dogs were anesthetized, intubated, and ventilated. The chest was shaved, prepped with betadine and the animal draped. Using sterile procedure, a thoracotomy was performed in the fifth intercostal space. The pericardium was incised, exposing the anterior surface of the left ventricle. This allowed visualization of the infarct as pale-yellow, non contracting tissue in the antero-apical wall of the left ventricle. Retrovirus was injected directly into the wall of the heart.

Retroviral supernatants of LNPOZ and LMDSN were mixed in equal volumes and injected through a 27 gauge needle into the visible infarct within an area of 1 cm^2. The location of the injections was marked with a suture. Three injections, each of 0.3 ml, were made per infarct. For injection of LNPOZ, viral supernatant was diluted 1:1 with growth medium and 0.3 ml of solution was injected at 3 sites within the infarcted area. After injection the chest was closed, air was evacuated from the chest, and the dogs were allowed to recover for 7-12 days. At 7-12 days the dogs were euthanized and hearts excised for analysis. Sections were obtained for immunostaining and histology.

This example was an attempt to convert to a skeletal muscle phenotype the cells present in a scar forming in the living animal after a myocardial infarction.

The density of dividing cells in a myocardial scar is at its peak 5-14 days after infarction and retroviruses preferentially transduce only proliferating cells. Accordingly, this time period was chosen to inject MyoD expressing retroviruses into a grossly infarcted segment of myocardium. Six dogs entered the study on MyoD gene transfer. As presented in Table 2, five of six dogs had developed gross myocardial infarctions when observed following thoracotomy at 6-11 days post coronary artery occlusion. Four dogs were injected with both LNPOZ and LMDSN. Two dogs (one of which was the dog that did not develop infarction) were injected with LNPOZ only.

To establish whether retroviral uptake was possible in the infarcted area and to localize precisely the injection site, the LNPOZ retrovirus carrying the β-galactosidase gene was injected. Seven to 12 days after injection of retrovirus into ischemic dog heart the area of gene transfer was confirmed by Xgal staining. 3 out of 4 dogs injected with both LNPOZ and LMDSN stained positively for β-galactosidase (Table 2). The one dog with an infarct that was injected with LNPOZ alone served as a control and also stained positive for β-galactosidase.

To determine whether expression of the MyoD gene in these injected animals resulted in conversion of cells to the skeletal muscle phenotype, frozen section were stained with an antibody specific to skeletal fast myosis heavy chain (α-MHC). Only in sections from β-galactosidase positive tissue of the 3 dogs injected with both LMDSN and LNPOZ were found multiple small clusters of cells that stained positive for skeletal fast MHC. These cells co-stained positive for the muscle marker α-actinin. No such cells were found in surrounding uninfarcted myocardium, in normal myocardium or in the cardiac scar of the animal injected with LNPOZ alone. Skeletal muscle from the diaphragm of one dog with the specific anti-α-MHC monoclonal antibody was also stained. As expected, the myofibers stained positively. Similar sections of normal myocardium do not react with this antibody.

These data indicate that a percentage of cells in the infarcted area injected with a MyoD expressing retrovirus underwent conversion to the skeletal muscle phenotype. The lower number of cells converted may be due to the fact that the

retrovirus was of a low titer or perhaps the period of time after gene transfer was not optimal for cellular conversion. It is likely that the cell type converted by MyoD was not the cardiomyocyte because such a severe infarct leads to extensive loss of cardiomyocyte viability. The fact that retroviruses preferentially infect dividing cells also points to non-myocytes as the target cells for conversion.

Tissue obtained from the infarcted areas of all dog hearts revealed loss of myocytes with infiltration of fibroblasts and mononuclear cells into the region. Picrosirius red staining revealed bright red areas of collagen deposition which were easily distinguished from surrounding viable myocytes at the edge of the infarct which stained yellow. No definite skeletal muscle myotubes were identified.

The high levels of gene expression obtained from DNA directly injected following 15 minutes of occlusion and reperfusion did not differ from levels obtained with sham operated rats. It is notable that expression of RSV-luciferase was increased following ischemia resulting from 1 hour occlusion. This observation suggests that ischemic injury may increase DNA uptake into cardiocytes, perhaps through alterations in cellular permeability. Such changes in cellular permeability following coronary artery occlusion have been reported [Harper, I.S. et al. (1989) *Basic Research In Cardiology*, **84**, 208-26]. Thus, foreign genes can be taken up by direct injection into ischemic/reperfused myocardium and transcribed by a strong promotor.

The lack of any skeletal MHC positive multinucleated myotubes in the injected tissue may have been due to the relatively low number of cells that were converted to the skeletal phenotype. It is likely, however, that with the use of retroviruses of a higher titer or of viral vectors such as adenovirus, a higher percentage of cardiac fibroblasts could be converted to the skeletal muscle phenotype *in vivo* and myotubes might be formed. It is also possible that the experiments underestimated the fraction of cells that took up the retrovirus and expressed MyoD. Expression of MyoD soon after uptake of the retrovirus might well lead to a rapid withdrawal from the cell cycle, allowing uninfected neighboring cells to selectively proliferate and greatly outnumber the transduced, MHC positive cells at the time of sampling 7-10 days after infection.

From the foregoing description, one skilled in the art can readily ascertain the essential characteristics of the invention and, without departing from the spirit and scope thereof, can adapt the invention to various usages and conditions. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient, and although specific terms have been employed herein, they are intended in a descriptive sense and not for purposes of limitation.

Table I
Effect of Ischemia/Reperfusion on Recombinant Protein Expression

15 Min	Non Ischemic		Ischemic	
	Animal*	Light Units	Animal	Light Units
	1	28522	12	3355
	2	4148	13	32432
	3	628	14	2024
	4	26361	15	3089
	5	575	16	416
	6	6928	17	3538
	7	7686	18	29640
	8	936	19	813
	9	154	20	535
			21	236
60 Minutes				
	Animal**			
	24	6597	30	246
	25	279	31	7183
	26	7700	32	43229
	27	4062	33	6080
	28	11104	34	22287
			35	524

*Two additional animals in each group had no detectable luciferase activity

**One additional animal in the Non Ischemic group had no detectable luciferase activity.

TABLE 2

Dog No.	Retrovirus Injection		Injection Protocol (interval in days)		Gross Infarct at time of injection	ß-gal	MHC	Actinin	COMMENT
	LNPOZ	LMDSN	Injection	Euthanized					
2952	+	+	10	12	+	-	N.D.	N.D.	Failed injection. No DNA uptake
2953	+	+	10	12	+	+	+	+	
2959	+	+	6	7	+	+	+	+	
3038	+	-	6	9	-	-	N.D.	N.D.	No infarct. No DNA uptake
3041	+	+	6	8	+	+	+	+	
3043	+	-	11	7	+	+	-	-	

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: UNIVERSITY OF SOUTHERN CALIFORNIA

(ii) TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR TRANSDUCTION OF CELLS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(E) COUNTRY: U.S.A.
(F) ZIP: 90012

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 29,215
(C) REFERENCE/DOCKET NUMBER: 1920-341

(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1785 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CTCCGGGACA TAGACTTGAC AGGCCCCGAC GGCTCTCTCT GCTCCTTTGA GACAGCAGAC 240

GACTTCTATG ATGATCCGTG TTTCGACTCA CCAGACCTGC GCTTTTTTGA GGACCTGGAC 300

CCGCGCCTGG TGCACGTGGG AGCCCTCCTG AAACCGGAGG AGCACGCACA CTTCTCTACT 360

GCGGTGCACC CAGGCCCAGG CGCTCGTGAG GATGAGCATG TGCGCGCGCC CAGCGGGCAC 420

CACCAGGCGG GTCGCTGCTT GCTGTGGGCC TGCAAGGCGT GCAAGCGCAA GACCACCAAC 480

GCTGATCGCC GCAAGGCCGC CACCATGCGC GAGCGCCGCC GCCTGAGCAA AGTGAATGAG 540

GCCTTCGAGA CGCTCAAGCG CTGCACGTCC AGCAACCCGA ACCAGCGGCT ACCCAAGGTG 600

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ACTCTGGCTG CACCTCACTG GGCCAGAGCT GATCCTTGAG TGGCCAGGCG CTCTTCCTTT 1440

CCTCATAGCA CAGGGGTGAG CCTTGCACAC CTAAGCCCTG CCCTCCACAT CCTTTTGTTT 1500

GTCACTTTCT GGAGCCCTCC TGGCACCCAC TTTTCCCCAC AGCTTGCGGA GGCCACTCAG 1560

GTCTCAGGTG TAACAGGTGT AACCATACCC CACTCTCCCC CTTCCCGCGG TTCAGGACCA 1620

CTTATTTTTT TATATAAGAC TTTTGTAATC TATTCGTGTA AATAAGAGTT GCTTGGCCAG 1680

AGCGGGAGCC CCTTGGGCTA TATTTATCTC CCAGGCATGC TGTGTAGTGC AACAAAAACT 1740

TTGTATGTTT ATTCCTCAAG CGGGCGAGTC AGGTGTTGGA AATCC 1785

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1571 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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AGCTTAAGCC CGGAAGCCCG AGGGCCCCTG GAAGAAAAGG GACTGGGGAC CCCTGAGCAT 300

TGTCCAGGCC AGTGCCTGCC GTGGGCATGT AAGGTGTGTA AGAGGAAGTC TGTGTCGGTG 360

GACCGGAGGA GGGCAGCCAC ACTGAGGGAG AAGCGCAGGC TCAAGAAAGT GAATGAGGCC 420

TTCGAGGCCC TGAAGAGGAG CACCCTGCTC AACCCCAACC AGCGGCTGCC TAAAGTGGAG 480

ATCCTGCGCC ATGCCATCCA GTACATTGAG CGCCTACAGG CCTTGCTCAG CTCCCTCAAC 540

CAGGAGGAGC GCGATCTCCG CTACAGAGGC GGGGGCGGGC CCAGCCCATG GTGCCCAGTG 600

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1296 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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AAATAGTCTT AGTACTTTCG TTTTTATGTA AACCTAAAGG ACATATTTTA AATGTGGAAT 1200

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TTCTACACGT AAAAAAAAAA AAAAAAAAAA AAAAAA 1296

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1432 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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CAGGGCTCAG ATGAGGACGA GCACGTGCGA GCGCCTACCG GCCACCACCA GGCTGGTCAC 240

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AAGAGGTGTA CCACGACCAA CCCCAACCAG AGGCTGCCCA AGGTGGAGAT CCTCAGGAAT 420

GCCATCCGCT ACATCGAGAG CCTGCAGGAG TTGCTGAGAG AGCAGGTGGA GAACTACTAT 480